

1
SEQUENCE LISTING

<110> SmithKline Beecham Corporation
Lambert, Millard H
Xu, Robert
Wisely, Bruce
Collins, Jon

<120> CAR LIGAND-BINDING DOMAIN POLYPEPTIDE CO-CRYSTALLIZED WITH A LIGAND, AND METHODS OF DESIGNING LIGANDS THAT MODULATE CAR ACTIVITY

<130> PR60235

<150> 60/488,415

<151> 2003-07-18

<160> 7

<170> PatentIn version 3.2

<210> 1

<211> 1450

<212> DNA

<213> *Homo sapiens*

<220>

<221> CDS

<222> (273)..(1316)

<400> 1
gtgagcttgc tccttaagtt acaggaactc tccttataat agacacttca ttttccttagt 60
ccatccctca tgaaaaatga ctgaccactg ctgggcagca ggagggatga taatcctaac 120
tccaaatcaact ggcaactcct gagatcagag gaaaaccagc aacagcgtgg gagtttgggg 180
agaggcattc cataccagat tctgtggcct gcaggtgaca tgctgcctaa gagaagcagg 240
agtctgtgac agccacccca acacgtgacg tc atg gcc agt agg gaa gat gag 293
Met Ala Ser Arg Glu Asp Glu
1 5
ctg agg aac tgt gtg gta tgt ggg gac caa gcc aca ggc tac cac ttt 341
Leu Arg Asn Cys Val Val Cys Gly Asp Gln Ala Thr Gly Tyr His Phe
10 15 20
aat gcg ctg act tgt gag ggc tgc aag ggt ttc ttc agg aga aca gtc 389
Asn Ala Leu Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Val
25 30 35
agc aaa agc att ggt ccc acc tgc ccc ttt gct gga agc tgt gaa gtc 437
Ser Lys Ser Ile Gly Pro Thr Cys Pro Phe Ala Gly Ser Cys Glu Val
40 45 50 55
agc aag act cag agg cgc cac tgc cca gcc tgc agg ttg cag aag tgc 485
Ser Lys Thr Gln Arg Arg His Cys Pro Ala Cys Arg Leu Gln Lys Cys
60 65 70
tta gat gct ggc atg agg aaa gac atg ata ctg tcg gca gaa gcc ctg 533
Leu Asp Ala Gly Met Arg Lys Asp Met Ile Leu Ser Ala Glu Ala Leu

75	80	85	2	
gca ttg cgg cga gca aag cag gcc cag cgg cgg gca cag caa aca cct Ala Leu Arg Arg Ala Lys Gln Ala Gln Arg Arg Ala Gln Gln Thr Pro 90	95	100		581
gtg caa ctg agt aag gag caa gaa gag ctg atc cgg aca ctc ctg ggg Val Gln Leu Ser Lys Glu Gln Glu Glu Leu Ile Arg Thr Leu Leu Gly 105	110	115		629
gcc cac acc cgc cac atg ggc acc atg ttt gaa cag ttt gtg cag ttt Ala His Thr Arg His Met Gly Thr Met Phe Glu Gln Phe Val Gln Phe 120	125	130		677
agg cct cca gct cat ctg ttc atc cat cac cag ccc ttg ccc acc ctg Arg Pro Pro Ala His Leu Phe Ile His His Gln Pro Leu Pro Thr Leu 140	145	150		725
gcc cct gtg ctg cct ctg gtc aca cac ttc gca gac atc aac act ttc Ala Pro Val Leu Pro Leu Val Thr His Phe Ala Asp Ile Asn Thr Phe 155	160	165		773
atg gta ctg caa gtc atc aag ttt act aag gac ctg ccc gtc ttc cgt Met Val Leu Gln Val Ile Lys Phe Thr Lys Asp Leu Pro Val Phe Arg 170	175	180		821
tcc ctg ccc att gaa gac cag atc tcc ctt ctc aag gga gca gct gtg Ser Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala Val 185	190	195		869
gaa atc tgt cac atc gta ctc aat acc act ttc tgt ctc caa aca caa Glu Ile Cys His Ile Val Leu Asn Thr Thr Phe Cys Leu Gln Thr Gln 200	205	210		917
aac ttc ctc tgc ggg cct ctt cgc tac aca att gaa gat gga gcc cgt Asn Phe Leu Cys Gly Pro Leu Arg Tyr Thr Ile Glu Asp Gly Ala Arg 220	225	230		965
gtg ggg ttc cag gta gag ttt ttg gag ttg ctc ttt cac ttc cat gga Val Gly Phe Gln Val Glu Phe Leu Glu Leu Leu Phe His Phe His Gly 235	240	245		1013
aca cta cga aaa ctg cag ctc caa gag cct gag tat gtg ctc ttg gct Thr Leu Arg Lys Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu Leu Ala 250	255	260		1061
gcc atg gcc ctc ttc tct cct gac cga cct gga gtt acc cag aga gat Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg Asp 265	270	275		1109
gag att gat cag ctg caa gag gag atg gca ctg act ctg caa agc tac Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser Tyr 280	285	290		1157
atc aag ggc cag cag cga agg ccc cgg gat cgg ttt ctg tat gcg aag Ile Lys Gly Gln Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala Lys 300	305	310		1205
ttg cta ggc ctg ctg gct gag ctc cgg agc att aat gag gcc tac ggg Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr Gly 315	320	325		1253

tac caa atc cag cac atc cag ggc ctg tct gcc atg atg ccg ctg ctc Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met Pro Leu Leu 330 335 340	3 1301
cag gag atc tgc agc tgaggccatg ctcacttcct tccccagctc acctggaaca Gln Glu Ile Cys Ser 345	1356
ccctggatac actggagtgg gaaaatgctg ggaccaaaga ttgggcccggg ttcaaaggga gcccagtgg tgcaatgaaa gactaaagca aaac	1416 1450
<210> 2 <211> 348 <212> PRT <213> Homo sapiens	
<400> 2	
Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp 1 5 10 15	Met Ala Ser Arg Glu Asp Glu Leu Arg Asn Cys Val Val Cys Gly Asp 1 5 10 15
Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys 20 25 30	Gln Ala Thr Gly Tyr His Phe Asn Ala Leu Thr Cys Glu Gly Cys Lys 20 25 30
Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro 35 40 45	Gly Phe Phe Arg Arg Thr Val Ser Lys Ser Ile Gly Pro Thr Cys Pro 35 40 45
Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro 50 55 60	Phe Ala Gly Ser Cys Glu Val Ser Lys Thr Gln Arg Arg His Cys Pro 50 55 60
Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met 65 70 75 80	Ala Cys Arg Leu Gln Lys Cys Leu Asp Ala Gly Met Arg Lys Asp Met 65 70 75 80
Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln 85 90 95	Ile Leu Ser Ala Glu Ala Leu Ala Leu Arg Arg Ala Lys Gln Ala Gln 85 90 95
Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu 100 105 110	Arg Arg Ala Gln Gln Thr Pro Val Gln Leu Ser Lys Glu Gln Glu Glu 100 105 110
Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met 115 120 125	Leu Ile Arg Thr Leu Leu Gly Ala His Thr Arg His Met Gly Thr Met 115 120 125
Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His 130 135 140	Phe Glu Gln Phe Val Gln Phe Arg Pro Pro Ala His Leu Phe Ile His 130 135 140
His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His 145 150 155 160	His Gln Pro Leu Pro Thr Leu Ala Pro Val Leu Pro Leu Val Thr His 145 150 155 160
Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr 165 170 175	Phe Ala Asp Ile Asn Thr Phe Met Val Leu Gln Val Ile Lys Phe Thr 165 170 175

Lys Asp Leu Pro Val Phe Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser
 180 185 190

Leu Leu Lys Gly Ala Ala Val Glu Ile Cys His Ile Val Leu Asn Thr
 195 200 205

Thr Phe Cys Leu Gln Thr Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr
 210 215 220

Thr Ile Glu Asp Gly Ala Arg Val Gly Phe Gln Val Glu Phe Leu Glu
 225 230 235 240

Leu Leu Phe His Phe His Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu
 245 250 255

Pro Glu Tyr Val Leu Leu Ala Ala Met Ala Leu Phe Ser Pro Asp Arg
 260 265 270

Pro Gly Val Thr Gln Arg Asp Glu Ile Asp Gln Leu Gln Glu Glu Met
 275 280 285

Ala Leu Thr Leu Gln Ser Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg
 290 295 300

Asp Arg Phe Leu Tyr Ala Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg
 305 310 315 320

Ser Ile Asn Glu Ala Tyr Gly Tyr Gln Ile Gln His Ile Gln Gly Leu
 325 330 335

Ser Ala Met Met Pro Leu Leu Gln Glu Ile Cys Ser
 340 345

<210> 3
 <211> 714
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (1)..(714)

<400> 3
 cct gtg caa ctg agt aag gag caa gaa gag ctg atc cgg aca ctc ctg 48
 Pro Val Gln Leu Ser Lys Glu Gln Glu Leu Ile Arg Thr Leu Leu
 1 5 10 15

ggg gcc cac acc cgc cac atg ggc acc atg ttt gaa cag ttt gtg cag 96

Gly Ala His Thr Arg His Met	Gly Thr Met	Phe Glu Gln Phe Val Gln	5
20	25	30	
Phe Arg Pro Pro Ala His Leu	Phe Ile His His Gln Pro Leu Pro Thr		144
35	40	45	
Leu Ala Pro Val Leu Pro Leu Val	Thr His Phe Ala Asp Ile Asn Thr		192
50	55	60	
Phe Met Val Leu Gln Val Ile Lys Phe Thr Lys Asp Leu Pro Val Phe			240
65	70	75	80
cgt tcc ctg ccc att gaa gac cag atc tcc ctt ctc aag gga gca gct			288
Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala			
85	90	95	
gtg gaa atc tgt cac atc gta ctc aat acc act ttc tgt ctc caa aca			336
Val Glu Ile Cys His Ile Val Leu Asn Thr Thr Phe Cys Leu Gln Thr			
100	105	110	
caa aac ttc ctc tgc ggg cct ctt cgc tac aca att gaa gat gga gcc			384
Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr Thr Ile Glu Asp Gly Ala			
115	120	125	
cgt gtg ggg ttc cag gta gag ttt ttg gag ttg ctc ttt cac ttc cat			432
Arg Val Gly Phe Gln Val Glu Phe Leu Glu Leu Leu Phe His Phe His			
130	135	140	
gga aca cta cga aaa ctg cag ctc caa gag cct gag tat gtg ctc ttg			480
Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu Leu			
145	150	155	160
gct gcc atg gcc ctc ttc tct cct gac cga cct gga gtt acc cag aga			528
Ala Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg			
165	170	175	
gat gag att gat cag ctg caa gag gag atg gca ctg act ctg caa agc			576
Asp Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser			
180	185	190	
tac atc aag ggc cag cag cga agg ccc cgg gat cgg ttt ctg tat gcg			624
Tyr Ile Lys Gly Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala			
195	200	205	
aag ttg cta ggc ctg ctg gct gag ctc cgg agc att aat gag gcc tac			672
Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr			
210	215	220	
ggg tac caa atc cag cac atc cag ggc ctg tct gcc atg atg			714
Gly Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met			
225	230	235	

<210> 4
 <211> 238
 <212> PRT
 <213> Homo sapiens
 <400> 4

Pro Val Gln Leu Ser Lys Glu Gln Glu Glu Leu Ile Arg Thr Leu Leu
1 5 10 15

Gly Ala His Thr Arg His Met Gly Thr Met Phe Glu Gln Phe Val Gln
20 25 30

Phe Arg Pro Pro Ala His Leu Phe Ile His His Gln Pro Leu Pro Thr
35 40 45

Leu Ala Pro Val Leu Pro Leu Val Thr His Phe Ala Asp Ile Asn Thr
50 55 60

Phe Met Val Leu Gln Val Ile Lys Phe Thr Lys Asp Leu Pro Val Phe
65 70 75 80

Arg Ser Leu Pro Ile Glu Asp Gln Ile Ser Leu Leu Lys Gly Ala Ala
85 90 95

Val Glu Ile Cys His Ile Val Leu Asn Thr Thr Phe Cys Leu Gln Thr
100 105 110

Gln Asn Phe Leu Cys Gly Pro Leu Arg Tyr Thr Ile Glu Asp Gly Ala
115 120 125

Arg Val Gly Phe Gln Val Glu Phe Leu Glu Leu Leu Phe His Phe His
130 135 140

Gly Thr Leu Arg Lys Leu Gln Leu Gln Glu Pro Glu Tyr Val Leu Leu
145 150 155 160

Ala Ala Met Ala Leu Phe Ser Pro Asp Arg Pro Gly Val Thr Gln Arg
165 170 175

Asp Glu Ile Asp Gln Leu Gln Glu Glu Met Ala Leu Thr Leu Gln Ser
180 185 190

Tyr Ile Lys Gly Gln Gln Arg Arg Pro Arg Asp Arg Phe Leu Tyr Ala
195 200 205

Lys Leu Leu Gly Leu Leu Ala Glu Leu Arg Ser Ile Asn Glu Ala Tyr
210 215 220

Gly Tyr Gln Ile Gln His Ile Gln Gly Leu Ser Ala Met Met
225 230 235

<211> 11
<212> PRT
<213> Artificial

<220>
<223> Amino acid sequence of an artificial His tag

<400> 5

Met Lys Lys Gly His His His His His Gly
1 5 10

<210> 6
<211> 69
<212> DNA
<213> Artificial

<220>
<223> Forward primer for amplifying amino acids 103-348, including sequences encoding a His tag, and including an NdeI restriction site

<400> 6
cggcggcgcc atatgaaaaa aggtcatcat catcatcatc atggcctgt gcaactgagt 60
aaggagcaa 69

<210> 7
<211> 45
<212> DNA
<213> Artificial

<220>
<223> Reverse primer for amplifying amino acids 103-348, incorporating a BamHI site

<400> 7
cggcggcgcg gatccttatt agctgcagat ctcctggagc agcgg 45